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VACCINATION OF MONKEYS WITH VIABLE *COCCIDIOIDES IMMITIS*: Control of Tissue Reactions by Prevaccination with Killed *C. immitis*

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ABSTRACT

Undesirable tissue reactions, resulting from the subcutaneous injection of 150 viable arthrospores of Coccidioides immitis (strain D-76), could be reduced by injecting formalin-killed arthrospores before injecting the viable organisms.

By the termination of the study, 6 and 12% of these vaccinated animals exhibited ulceration and lymphadenopathy, respectively, as compared with 100 and 83% of animals receiving only the viable vaccine. Agar/gel immunodiffusion precipitin titers of approximately 1:64 were evident 3 months after vaccination in animals receiving both vaccines as compared with 1:128 in those injected with the viable vaccine only.

The data indicated that the somatic reactions resulting from injection of a viable vaccine could be eliminated by preinjection of a killed vaccine. Although the tissue reactions were reduced by this treatment, respiratory challenge (7,500 strain Cash arthrospores) six months after vaccination indicated that the protective effect of the viable vaccine was also impaired. All animals receiving both vaccines developed mild pulmonary coccidioidomycosis, whereas only 50% of the animals receiving the viable vaccine only were infected. In addition, the group receiving both vaccines demonstrated a more rapid and higher postchallenge precipitin titer.

All vaccinated animals (those receiving the killed, the viable, or a combination of the two vaccines) survived for 4 months after challenge, as compared with 88% mortality (50% within 14 days) in the nonvaccinated controls.

I. INTRODUCTION

Second infections with Coccidioides immitis rarely, if ever, occur in nature,[1] and reports indicate that naturally acquired, primary, cutaneous coccidioidomycosis in man does not disseminate beyond the regional lymph nodes.[2-6] Previous reports from our laboratory have shown that protection against pulmonary challenge with C. immitis could be obtained in monkeys* by the subcutaneous injection of 10 viable arthrospores and that dissemination beyond the regional lymph nodes did not occur with a vaccine dose of this size.[7] Also, various strains of C. immitis, when used as a viable vaccine, exhibited substantial differences in side effects of inoculation[8] (ulcerated vaccination site and lymphadenopathy); these side effects could be controlled by oral amphotericin B therapy at the time of vaccination.[8] The present study was an attempt to control the side effects of the viable vaccine by preinjection of a killed vaccine. We hoped that this could be accomplished without impairing the protective effect of the viable vaccine.

II. METHODS AND RESULTS

The killed vaccine consisted of strain Cash arthrospores killed with 0.5% formalin, washed and resuspended in incomplete Freund's adjuvant (without TB organisms). A total of 36 mg of this preparation was injected subcutaneously at intervals of 0, 2, 6, and 10 weeks at various sites.

The viable vaccine consisted of 150 viable arthrospores of strain D-76, subcutaneously injected in the right forearm 30 days after the last injection with the killed preparation. Strain D-76 was selected for its ability to produce a pronounced somatic reaction. The large vaccine dose was used to insure ulceration and lymphadenopathy upon injection.

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* In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

The two principal groups of monkeys in this experiment (groups 1 and 2) received either the viable vaccine alone, or the killed vaccine followed in 30 days by the viable vaccine (Table 1). Six months after administration of the viable vaccine, these two groups, plus two groups of control animals (group 3 vaccinated with the killed product and group 4 nonvaccinated) were challenged via the respiratory route with approximately 7,500 arthrospores of strain Cash.

Two other control groups (5 and 6) received the viable vaccine with and without the killed vaccine, were not challenged but were maintained for histological comparison.

All animals were observed clinically throughout the period of vaccination and for 4 months following respiratory challenge. Gross and histopathological studies were made upon necropsy at the end of the experiment. Tissues for these studies were stained with the Giemsa, Gomori silver methenamine, or Ziel-Nielson acid-fast stains.

Of the animals receiving both viable and killed vaccines 6% exhibited ulceration and 12% showed lymphadenopathy, as compared with 100 and 83% in those receiving only the viable vaccine; this indicated substantial control of somatic reaction of the viable vaccine by preinjection of killed vaccine (Table 2).

Control of the viable vaccine by the killed vaccine was also indicated by the serological response of these animals 3 months after vaccination (Table 3). The horizontal line indicates the spread of the titers of monkeys in each group; the cross-hatch shows the mean titer for the group. The highest titer developed in animals receiving the viable product, the lowest in the group given the killed vaccine, and intermediate titers in those receiving both vaccines.

TABLE 1. PROTOCOL OF EXPERIMENT

	Group[a] 1	2	3	4	5	6
Vaccine	Viable	Killed and Viable	Killed	None	Killed and Viable	Viable
				None		
Resp. Challenge arthrospores	7,500	7,500	7,500	7,500	None	None

a. Clinical observations included x-ray, serology, gross pathology, histopathology, and culturing of necropsied organs.

TABLE 2. CONTROL OF SOMATIC REACTION TO VIABLE VACCINE

Immunization	Tissue Reaction To Viable Vaccine, No. reacting/No. vaccinated: Ulcerated Vaccination Site	Axillary Lymphadenopathy
Formalin-Killed Vaccine[a] and Viable Vaccine[b]	1/17 (6%)	2/17 (12%)
Viable Vaccine Alone	12/12 (100%)	10/12 (83%)

a. Total dose: 36 mg (0, 2, 6, and 10 wks).
b. 150 viable arthrospores: strain D-76.

TABLE 3. PRECHALLENGE TITERS (FROM VACCINE)

Vaccine	Agar/Gel Immunodiffusion Precipitin Titer 1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
Viable					———	———		
Killed and Viable			———	———	———	———		
Killed	———	———	———					

Six months after the last vaccination the animals received a respiratory challenge of approximately 7,500 arthrospores. Two facts are evident from the data in Table 4 which can later be correlated with the histological findings: The higher the prechallenge titer (shown in the 0 column), the slower the development of the disease, as reflected by the acceleration of the postchallenge titer. The lower the prechallenge titer, the greater the maximum titer developed after challenge. Note that the group receiving the viable vaccine only maintained its prechallenge titer until 60 days postchallenge, in contrast to the immediate rise in titer of the other two groups.

TABLE 4. MEAN POSTCHALLENGE TITER

Vaccine	Days After Respiratory Challenge					
	0	15	45	60	90	120
Viable	1:64	1:64	1:64[a]	1:256[a]	1:512[a]	1:256
Killed and Viable	1:16	1:256[a]	1:512[a]	1:1024	1:2048	1:2048
Killed	1:2	1:256[a]	1:1024[a]	1:4096[a]	1:2048	1:2048[a]

a. Slight or incomplete reaction.

Table 5 shows the clinical record of all challenged animals during the subsequent 4-month observation period. All animals receiving the viable vaccine remained in good flesh and healthy in appearance and actions throughout the observation period.

Some apathy and slight loss of appetite were noted among those receiving only the killed vaccine, but otherwise they appeared healthy. All of the 24 vaccinated animals survived the respiratory challenge. This was in striking contrast to the evidence of extremely severe illness in the nonvaccinated control animals. That group suffered an 88% mortality during the 4-month period; 50% of them died by the 14th day postchallenge.

Note the inverse relationship between the height of serological titer before and after challenge. The value shown for the postchallenge titer of the control group is the 90-day titer of the one surviving animal because most of the controls died before high titers became evident.

The mortality and serological record of the nonvaccinated controls (Table 6) indicates a direct relationship between length of survival and height of titer. Note also, that 3 of the 4 animals tested on the 15th day postchallenge had developed titers of from 1:4 to 1:16.

TABLE 5. CLINICAL RECORD FOLLOWING RESPIRATORY CHALLENGE

Vaccine	Clinical Observation	Average Maximum Titer		Mortality, dead/total
		Prechallenge	Postchallenge	
Viable	Healthy	1:128[a]	1:1024[a]	0/9
Killed and Viable	Healthy	1:164[a]	1:4096[a]	0/8
Killed	Some loss of appetite, but healthy in appearance.	1:16[a]	1:4096[a]	0/7
None (Controls)	Loss of weight, extreme debilitation, coughing, rapid respiration.	Neg.	1:16,384[b]	7/8

a. Slight or incomplete reaction.
b. The one surviving control.

TABLE 6. SEROLOGICAL RECORD OF NONVACCINATED CONTROLS FOLLOWING RESPIRATORY CHALLENGE

Animal Number	Immunodiffusion Titers On Days Indicated										
	11	14	15	20	39	42	49	53	60	90	120
7C-23	Died										
T-61	Died										
7C-21		Neg Died									
T-66		Hemolyzed Died									
T-65			1:8	Hemolyzed Died							
T-68			1:16		1:512 Died						
T-27			Neg			1:2048[a]	1:1024	1:1024 Died			
6C-17			1:4			1:1024	1:4096		1:2048	1:16,380	1:2048[a]

a. Slight or incomplete reaction.

In general, the histological findings on autopsy correlated with the serological picture and with clinical signs and symptoms (Table 7). The severity of the disease increased stepwise in groups 1 through 4, as indicated by clinical symptoms, mean maximal serological titer, and extent of pathological involvement of the lungs.

Although the mean maximal serological titer was the same (1:4096) for groups 2 and 3, the titers increased at a faster rate in group 3. Approximately half of the animals in group 1 were not infected (all animals in groups 2, 3, and 4 developed pulmonary coccidioidomycosis), although the extent of lung involvement of infected animals in groups 1 and 2 is shown to be equal (+).

A comparison of all animals that received the viable vaccine and were later challenged via the respiratory route (17 monkeys) with those maintained as unchallenged vaccine controls (13 monkeys) disclosed minor dissemination (such as involvement of inguinal, splenic, or pancreatic lymph nodes) in 3 animals of each group. One of the 3 animals in the latter group (unexposed vaccine controls) exhibited generalized infection. The 3 disseminations in this group are thought to have been due to the large viable-vaccine dose (150 spores) and the high relative virulence of the vaccine strain used (D-76).

TABLE 7. CORRELATION OF DATA AFTER RESPIRATORY CHALLENGE

Group	Vaccine	Clinical Symptoms	Mean Maximal Serological Titer	No. Deaths/ No. Challenged	Lung Pathology[a]
1	Viable	None	1:1024[b]	0/9	+[c]
2	Killed + Viable	None	1:4096[b]	0/8	+
3	Killed	Apathy Loss of Appetite	1:4096[b]	0/7	++
4	None	Wt. Loss, Coughing, Rapid Respiration, Extreme Debilitation,	1:16,384	7/8	++++

a. Relative extent of lung involvement (+, minimal; ++, moderate; ++++, severe).
b. Slight or incomplete reaction.
c. This group contained 4 negative and 1 questionable animal.

III. CONCLUSIONS

It is evident from the data presented that the invasiveness of the viable vaccine can be arrested, by preinjection of a killed vaccine, to such an extent that the untoward tissue reactions do not occur. However, under the conditions of this experiment (the extremely large challenge dose), it appears that the development of immunity was also impaired to a certain extent. Although the infectious dose of C. immitis received in nature is unknown, it is logical to believe that it would be more on the order of 2 or 3 organisms to possibly 50 organisms, rather than 7,500 arthrospores as used in this experiment.

It is hoped that a repetition of the experiment, with a more realistic challenge dose (50 to 100 organisms) and a lower viable-vaccine dose (10 organisms) of a milder vaccine strain will prove to be more successful.

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